Males in Rural Bangladeshi Communities Are More Susceptible to Chronic Arsenic Poisoning than Females: Analyses Based on Urinary Arsenic

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Spot urine samples were collected from the inhabitants of two rural communities in northwestern Bangladesh. We compared arsenic levels in the urine samples ($[As]_u$; $n = 346$) with those in water from tube wells ($[As]_{tw}$; range < 1–535 µg/L; $n = 86$) on an individual basis. The small variation of $[As]_u$ within subjects and highly positive correlation with $[As]_{tw}$ indicate that $[As]_u$ is a useful indicator of exposure. Analyses of $[As]_u$ showed that creatinine correction was necessary, that $[As]_u$ only reflected recent exposure, and that there were substantial interindividual differences for a given $[As]_{tw}$ level. To evaluate the toxic effects of arsenic exposure, we constructed a system for rating skin manifestations, which revealed distinct sex-related differences. Comparison of males and females in the same households confirmed that skin manifestations were more severe in the males, and in the males of one community a dose–response relationship between $[As]_u$ and the degree of skin manifestation was evident. The results of this study indicate that $[As]_u$ in spot urine samples can be used as an exposure indicator for As. They suggest that there might be sex-related, and perhaps community-related, differences in the relationship between $[As]_u$ and skin manifestations, although several confounding factors, including sunlight exposure and smoking habits, might contribute to the observed sex difference. The existence of such differences should be further confirmed and examined in other populations to identify the subpopulations sensitive to chronic arsenic toxicity. *Key words*: Bangladesh, chronic arsenic toxicity, dose–response relationship, groundwater contamination, keratosis, melanosis, urinary excretion. *Environ Health Perspect* 109:1265–1270 (2001). [Online 28 November 2001]
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Large-scale human exposure to arsenic through contaminated groundwater is a serious health threat in many Asian and Latin American countries. With the exception of a series of studies in Taiwan (*1–5*), attention has only recently been given to the epidemiological and human toxicological aspects of this contamination. The tube wells that provide drinking water in rural Bangladesh are contaminated with geologically derived arsenic (*6*). Consumption of the contaminated water is a likely cause of skin conditions such as keratosis and melanosis, which are sensitive manifestations of chronic arsenic toxicity, in many members of these communities.

In this paper, we describe the dose–response relationship of chronic arsenic exposure and skin problems in rural Bangladeshi communities. Two methodologic features distinguish this investigation from other recently published reports of the arsenic problem in Bangladesh and the nearby region of West Bengal, India (*7–13*).

First, the selected indicator of dose/exposure is urinary arsenic concentration ($[As]_u$). This contrasts with other studies that relied on the arsenic concentration in the water from tube wells ($[As]_{tw}$) (*14,15*), or indices derived from it (*9,12*), as the dose/exposure indicator. Use of $[As]_{tw}$ or related indices assumes that water intake across individuals is similar, or relies on an estimated water intake. The use of biological dose indicators is more precise, but has been limited by a paucity of reports applying such indicators to the ingestion of arsenic-contaminated water. Recently, a good correlation between $[As]_u$ and arsenic concentrations in water or soil was reported for apparently healthy populations in the United States (*16,17*). A study on the arsenic-exposed population in West Bengal, India reported on $[As]_u$ distribution in the affected population, although a relationship with arsenic-induced effects was not reported (*8*). In addition, a recent study on a Bangladeshi population used $[As]_u$ to show an elevated risk for arsenicosis in the subpopulation with the highest quartile $[As]_u$ values (*7*). The small sample size limited the significance of the study, and an elevated risk was only recognized in the highest quartile group.

Second, we used a graded scoring of skin manifestation as the effect indicator. Nearly all of the preceding studies used a differential diagnosis approach for defining the end point. Diagnoses of the features characteristic of arsenic-induced skin manifestations were made by experienced personnel in some cases (*9,12*) and by consensus diagnostics among two or more examiners (usually physicians) in other cases (*7,18*). These approaches per se suggest the difficulty of diagnosing arsenic-induced skin manifestations, which vary from one person to another in terms of their severity (*19*). Instead of relying on such differential diagnosis, which generates binary data, we relied on graded scores based on physicians' inspections, regardless of the etiology [i.e., whether caused by arsenic or by some other agent(s)].

Our study using the new methodologies examined a much larger sample size than the previous Bangladeshi study (*7*). We first evaluated the appropriateness of using $[As]_u$ as a dose/exposure indicator by examining the dose–response relationship between $[As]_u$ and $[As]_{tw}$. Then, we examined possible factors that modify the toxic manifestations of chronic arsenic exposure. Our study was reinforced with repeated observations of the same individuals and used a within-household male–female comparison to specifically address the issue of sex-related differences in susceptibility to arsenic toxicity. In particular, we focused on the effects of sex, area, and nutritional status (i.e., body fat), which are possible modulators of arsenic toxicity (*5,7,9,12,18,20*). The tube wells in the subject villages typically supplied drinking water to one or several households. Although the tube wells were in close proximity, $[As]_{tw}$ showed wide variation, enabling determination of a dose–response relationship in a relatively homogenous environmental setting.

Methods

Study areas and populations. The study areas were two rural communities (SV and SP) in Sibganji thana (prefecture), Nawabganj district, northwestern Bangladesh. The two communities, each about 1 km² in area, are 5 km apart.

The inhabitants of these villages subsist on paddy cultivation and derive some income from selling mangoes as a cash crop. Their water supply is entirely dependent on tube wells that were installed either by households or by the government. Approximately 100 tube wells, with diverse arsenic concentrations, are used for drinking and cooking purposes.

According to our survey, SV contained 199 households with 933 inhabitants (481 males and 452 females) and SP contained 150 households with 747 inhabitants (405 males and 342 females).

Health examination. A pilot survey was conducted in November 1998. Urine samples were collected from a limited number of people ($n = 40$; 10 males and 10 females from each community). The main survey, conducted in February and March of 1999, included anthropometric measurements (height, weight, and four skinfold thicknesses: biceps, triceps, subscapular, and supra-iliac), collection of spot urine samples, and clinical skin examination by a dermatologist. Participation by inhabitants visiting health examination stations was voluntary. Data were collected from inhabitants who were at least 20 years of age (112 males and 138 females in SV; 108 males and 193 females in SP). This age group represented a similar proportion in each community, but represented more females (approximately 80% of the total adult female population) than males (approximately 50%).

All the procedures associated with the survey were approved by the Ethics Committee of the Graduate School of Medicine, The University of Tokyo. Written, informed consent was obtained from each participant.

Dermatologic examination and scoring of symptoms. The two dermatologists who conducted the dermatologic examinations were blind to the exposure conditions. They examined and scored skin manifestations, evaluating keratosis on the soles of the feet and palms of the hands and melanosis on the trunk. Keratosis was defined as skin lesions with at least five elevated small nodules or pits. These small nodules or pits were disseminated in the soles or palms bilaterally, having a cornlike, usually symmetrical shape, with a mean diameter of about 5 mm. We arbitrarily set a minimum of five nodules and pits to exclude small numbers produced by simple mechanical stimulation (such as walking barefoot and manual agricultural labor).

Melanosis including hypopigmentation was identified as poorly demarcated, mottled areas, recognizable on most areas of the abdomen and back, which are relatively unexposed to sunlight. Melanosis can be distinguished from melanoma by its relatively homogeneous size and shape and its multiple occurrence. Keratosis on the sole was scored from 0 (normal) to 4 (most severe), and that on the palm was scored from 0 to 3. Melanosis on the trunk was scored from 0 to 2. More details of the dermatologic findings will be reported elsewhere (*21*). Assuming that early manifestations of poisoning are more frequent than advanced manifestations, the resulting possible score of 9 for positive manifestations (4 + 3 + 2 for sole, palm, and trunk, respectively) was integrated into a single effect indicator called the dermatologic stage (DS; Table 1).

To determine the DS, we first ranked each of these manifestations in order of decreasing prevalence. Thus, the most prevalent manifestation was ranked 1 and the rarest ranked 9; skin of normal appearance was given a rank of 0. Prevalence was cumulative: if a person had a sole keratosis score of 3, then he/she was counted as positive for scores 1 and 2 as well. The highest rank of the sole, palm, and trunk scores was the individual's overall rank. The nine ranks were then collapsed into five stages, and the stage corresponding to the individual's rank was defined as his or her DS. The scoring was irrespective of the etiology (related to arsenic or not), with three exceptions. Calluses visually identified as resulting from physical labor and hereditary keratoderma or Darier's disease, identified by visual inspection and family history, were excluded.

Tube well survey. Water samples were collected from all the tube wells ($n = 101$) in SV and SP. Of these, 88 tube wells (32 in SV, 56 in SP) were used for drinking and cooking by the subjects of this study. Freshly pumped water was collected after at least 10 strokes of a hand pump. Each water sample was immediately acidified by adding HCl to a final concentration of 1% (v/v) to prevent

the precipitation of an iron complex that absorbs arsenic (*11*). At the time of water collection, the local health staff interviewed the residents to identify the depth and age of each tube well and the users of the well. The ages of the tube wells ranged from < 1 year to 30 years (median: 6 years).

Both the acidified water and the urine samples were kept frozen until they were taken to the laboratory in Japan, where they were kept at –80°C until the assay.

Arsenic determination. The total arsenic concentrations of the tube well water ($[As]_{tw}$) and urine samples ($[As]_u$) were determined by an atomic absorption spectrometer equipped with a flow injection hydride generator (HGAAS; ZL-4100, Perkin Elmer, Norwalk, CT, USA). The urine samples were first wet-ashed by heating with a mixture of nitric, perchloric, and sulfuric acids. Then both the water and the ashed urine samples were pre-reduced by potassium iodide in the acidified solution. Arsenic determination followed.

The detection limits (DLs) of the HGAAS were 1 and 3 µg/L in the water and urine samples, respectively. All of the urine samples produced values above the DL. Those water samples for which readings were below the DL were assigned a value of one-half the DL (0.5 µg/L).

Assay accuracy was ensured by the inclusion of reference materials: NIST 1643d (trace metal in water; National Institute of Standards and Technology, Gaithersburg, MD, USA) and NIES #18 (human urine; National Institute for Environmental Studies, Tsukuba, Japan) (*22*). The obtained values fell within the certified ranges (56.02 ± 0.73 µg/L for NIST 1643d, and 0.137 ± 0.011 mg/L for NIES #18). For the spot urine samples, the creatinine concentration was also determined spectrophotometrically using a commercial kit (Creatinine Wako, Wako Pure Pharmaceuticals, Osaka, Japan), based on the Jaffe's reaction (*23*).

Exposure indicators. $[As]_{tw}$ and $[As]_u$ were used as indicators of exposure to inorganic

Table 1. Classification criteria of DS and distribution of subjects by DS.

DS	Rank	Criteria for the stage[a] Manifestation	Prevalence [%][c]	Frequency distribution[b] No. (total = 468)	Percent of total
0		None		213	45.5
1	1	Sole 1	53.8	148	31.6
2	2	Sole 2	18.4	37	7.9
3	3	Palm 1	9.8	43	9.2
	4	Trunk 1	8.3		
4	5	Sole 3	5.1	19	4.1
	6	Palm 2	3.4		
5	7.5	Sole 4	1.1	8	1.7
	7.5	Trunk 2	1.1		
	9	Palm 3	0.6		

[a]For an individual diagnosed as sole = 3, palm = 2, trunk = 1, the corresponding ranks are 5, 6, and 4, respectively; the DS corresponding to the highest of these three ranks (6), which is 4, is assigned as this individual's DS (see text for details). [b]Actual distribution of the subjects classified for each DS. [c]Prevalence of the manifestation.

arsenic. Unless otherwise described, the $[As]_u$ was adjusted for creatinine (i.e., μg/g creatinine). Because the study population subsisted mainly on local produce and rarely consumed saltwater fish or other seafood, the contribution of these arsenic-rich foods to $[As]_u$ was assumed to be negligible. This notion was supported by a preliminary analysis using HPLC-inductively coupled plasma-mass spectrometry (kindly performed by Y. Shibata, National Institute for Environmental Studies, Tsukuba, Japan; data not shown). A peak corresponding to arsenobetaine, an organic form of arsenic commonly found in seafood, was absent from all 20 urine samples randomly selected from the study population; concurrently analyzed urine samples obtained from Japanese volunteers contained arsenobetaine.

Statistical analyses. Subjects were divided into four subgroups by sex and area (SP or SV). For $[As]_{tw}$, $[As]_u$, and percent body fat, a log-transformation was applied to normalize the distribution. In these cases, geometric mean and SD are shown. Between-group differences were tested by analysis of variance (ANOVA) or analysis of covariance (ANCOVA) using a JMP software program (version 4.0; SAS Institute, Cary, NC, USA). The associations between the exposure indicators, $[As]_{tw}$ and $[As]_u$, and the effect indicator, DS, were evaluated using the log-likelihood chi-square test. Unless otherwise specified, p-values < 0.05 were considered statistically significant.

Results

Tube well and urinary arsenic. The arsenic exposure indices, along with several basic characteristics of the subpopulations, are shown in Table 2. All of the subgroups had similar mean ages, and the expected sex differences for height, weight, and percent body fat were apparent. Twenty-eight percent of SP and 39% of SV subjects used tube wells in which $[As]_{tw}$ exceeded 50 μg/L, the regulatory upper limit for drinking water in Bangladesh. The mean $[As]_{tw}$ differed significantly between SV and SP, although the ranges were almost identical (SV, < 1–535 μg/L; SP, < 1–519 μg/L). The $[As]_u$ varied considerably among the subjects, ranging from 42 to 2,017 μg As/g creatinine in SV and from 24 to 3,398 μg As/g creatinine in SP. Significant area differences and marginal sex differences were evident for $[As]_u$, with females having higher levels than males.

Within-subject chronologic variation of $[As]_u$ was assessed in 21 individuals by collecting samples in November 1998 and February 1999 (Figure 1). Excluding two individuals described subsequently, the overall correlation of two $[As]_u$ was 0.89 (n = 19; p < 0.001), and the mean of the second $[As]_u$ was 106 ± 53% of the first, ranging between 56 and 265%. Two of the 21 individuals made a new tube well immediately after the first survey, due to the high level of $[As]_{tw}$ found in the old tube well. Their $[As]_u$ declined rapidly, to 27% and 18% of their first $[As]_u$ readings, respectively.

Relationship between $[As]_{tw}$ and $[As]_u$. Highly significant correlations (R^2 = 0.504; p < 0.001) were found between $[As]_{tw}$ and $[As]_u$ for the entire population (Figure 2) and for each of the four subgroups. ANCOVA, taking $[As]_{tw}$ as the covariate, showed that the effect of sex, where females had a higher $[As]_u$ than males, was significant (p < 0.005) after adjusting for $[As]_{tw}$, but the effect of area was not.

In the stepwise multiple regression analyses for $[As]_u$, $[As]_{tw}$ was selected with high significance in all the groups (Table 3). Although the age and percent body fat were significant or marginally significant in some cases, the observation did not suggest a pattern that was consistent across the subgroups. When both areas were combined, $[As]_{tw}$, age, and percent body fat were all selected as significant variables in males (both age and percent body fat were negative coefficients), while $[As]_{tw}$ and percent body fat (with a negative coefficient) were selected in females (data not shown).

Dose–response relationship between exposure indicators and skin manifestations. To examine the relationship between the exposure indicators and the effect indicator, DS, we divided the whole population into tertiles either by $[As]_u$ or $[As]_{tw}$ values and compared the frequency distributions of the DS in each tertile group (Figure 3). The $[As]_u$ values of the lower, middle, and upper tertile groups
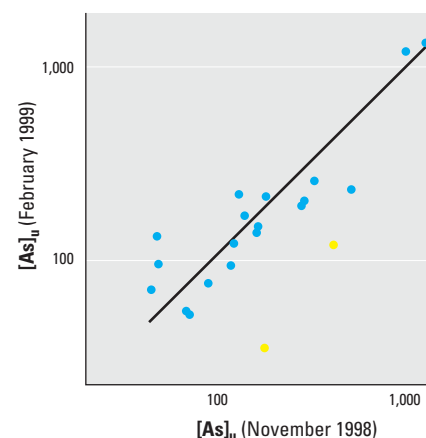

Figure 1. Comparison of $[As]_u$ values obtained from the same individuals at an interval of approximately 3 months. $[As]_u$ values measured on February 1999 are plotted against those measured on November 1998 (logarithmic scale). Each circle denotes one individual. The two yellow circles are individuals who showed a rapid decrease in $[As]_u$ after switching from high-As tube wells to low-As tube wells after the first sampling (November 1998) (see text). The straight line indicates x = y.
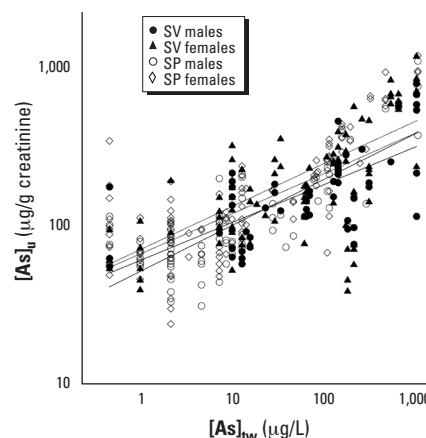

Figure 2. Relationship between $[As]_u$ and $[As]_{tw}$. Each circle denotes one individual, and the lines are the regression lines for each subpopulation. Females had a significantly higher $[As]_u$ than males, after correcting for $[As]_{tw}$ (by ANCOVA; see text). Note that the two dotted lines for females are higher than the two lines for males.

Table 2. Age, nutritional status, and arsenic exposure in the groups divided by sex and by area.

Area, sex	Age (years)	Height (cm)	Body weight (kg)	Percent body fat[a]	$[As]_{tw}$[b] (μg/L)	$[As]_u$ (μg/g creatinine)
SV						
Male	36 (1.5) (n = 112)	162 (5) (n = 111)	52 (7) (n = 111)	10.7 (1.4) (n = 111)	28 (6.0) (n = 104)	204 (2.1) (n = 64)
Female	36 (1.4) (n = 138)	151 (6) (n = 137)	43 (6) (n = 138)	21.4 (1.2) (n = 137)	26 (6.6) (n = 126)	219 (2.4) (n = 108)
SP						
Male	40 (1.5) (n = 108)	164 (6) (n = 107)	51 (7) (n = 100)	10.7 (1.5) (n = 108)	11 (6.6) (n = 108)	126 (2.3) (n = 69)
Female	35 (1.5) (n = 193)	152 (5) (n = 192)	44 (7) (n = 162)	20.9 (1.3) (n = 192)	10 (6.4) (n = 189)	174 (2.5) (n = 121)
ANOVA[c]						
Area	NS	#	NS	NS	**	**
Sex	#	**	**	**	NS	#
Area × sex	#	NS	NS	NS	NS	NS

NS, not significant (p > 0.1). Values shown are geometric mean (SD).
[a]Calculated from skinfold thickness. [b]The arithmetic means ± SDs were 88 ± 13, 85 ± 12, 58 ± 12, and 48 ± 8 μg/L for SV males, SV females, SP males, and SP females, respectively. [c]Two-way ANOVA using log-transformed variables. **p < 0.001; #0.05 < p < 0.1.

ranged from 24 to 110 (median = 76; n = 121), 111 to 235 (median = 155; n = 120), and 239 to 3,398 (median = 402; n = 121) μg/g creatinine, respectively. The corresponding $[As]_{tw}$ values ranged from 0.5 to 126 (median = 4), 0.5 to 519 (median = 11), and 0.5 to 535 (median = 88) μg/L for the respective groups. For each group defined by sex and village, these three tertile groups did not differ in age and percent body fat, except for the SP females, in which the lower tertile had a higher percent body fat. In the SV males, the $[As]_u$ tertile populations had different DS distributions from each other: the higher $[As]_u$ tertile groups had a greater proportion of individuals with a high DS (Figure 3A; χ^2 = 20.87; $p<0.05$ by likelihood ratio test; also see Figure 3C). Neither the SV females (Figure 3B; also see Figure 3D) nor the SP male–female groups (data not shown) showed this dose–response relationship. No such association was evident when the $[As]_{tw}$ tertiles replaced the $[As]_u$ tertiles (data not shown).

Although the levels of exposure were similar between the sexes, overall DS was higher in males than in females, and the DS of the two sexes were statistically different by a median test ($p < 0.005$).

Male–female comparison within the same households. To further confirm the possible sex difference in the manifestation of chronic arsenic toxicity noted above, we analyzed the $[As]_u$ values of male–female pairs from the same household who shared the same tube well. To avoid a possible confounding effect of age, we included in this analysis only male–female pairs whose age difference did not exceed 20 years. Of the 68 pairs (44 from SP and 24 from SV) who fit the criteria, $[As]_u$ values were highly correlated with the partner's $[As]_u$ ($R^2 = 0.74$; $p<0.001$; Figure 4). In most cases, the female's $[As]_u$ exceeded that of the male partner, and the male–female difference was significant ($p < 0.01$, paired t-test). On the other hand, males exceeded females in terms of DS in most couples, and the difference was significant ($p < 0.001$; paired t-test; data not shown).

Discussion

In this study we evaluated exposure to arsenic and manifestations of arsenic toxicity. To our knowledge, this is the first report to show a sex difference in the dose–response relationship with respect to $[As]_u$. Before exploring this point, we discuss the nature of $[As]_u$ as a dose indicator.

Urinary arsenic as a dose indicator. The study revealed the usefulness, and some limitations, of $[As]_u$ as an exposure indicator. Two observations that strongly support the use of $[As]_u$ as an exposure indicator in nonoccupational, chronic exposure to arsenic are the relatively small within-subject

variation of $[As]_u$, which is consistent with previous reports on nonoccupational populations (*16,17*), and the reasonable correlation with $[As]_{tw}$ for all four subgroups, as well as for male–female pairs using the same tube well. The latter observation implies that extra water intake of arsenic is negligible or constant across individuals, which will be discussed later. $[As]_u$ is especially valuable as an exposure indicator because spot urine is relatively easy to collect and transport, and it provides an objective measure of exposure.

In our samples, $[As]_u$ (corrected for creatinine) was judged a better indicator than the uncorrected urinary arsenic concentration.

Without this adjustment, the correlation between urinary arsenic and $[As]_{tw}$ and the within-household and intraindividual correlations of urinary arsenic, all of which are biologically reasonable correlations, were less remarkable (data not shown). Moreover, the partial correlation between uncorrected $[As]_u$ and creatinine was highly significant, even after being corrected for $[As]_{tw}$, while the partial correlation between the corrected $[As]_u$ and creatinine was not significant. Only 6.4 % (23/362) of the urine samples used for the analyses had a creatinine concentration < 0.3 g/L, while none exceeded 3.0 g/L, and exclusion of these samples did not change the

Table 3. Stepwise multiple regression analyses of urinary arsenic excretion (parameter estimate and its significance).

Area, sex	Adjusted R^2	Intercept	$[As]_{tw}$	Age	Percent body fat
SV					
Male	0.47	3.486**	0.294**	−0.293[a,#]	
Female	0.43	2.839**	0.320**		
SP					
Male	0.64	3.515**	0.356**		−0.237#
Female	0.51	3.482**	0.328**		−0.440*

[a]A blank indicates that the parameter was not selected (entered) as a significant independent variable in the regression model. **$p < 0.001$; *$p < 0.05$; #$0.05 < p < 0.1$.
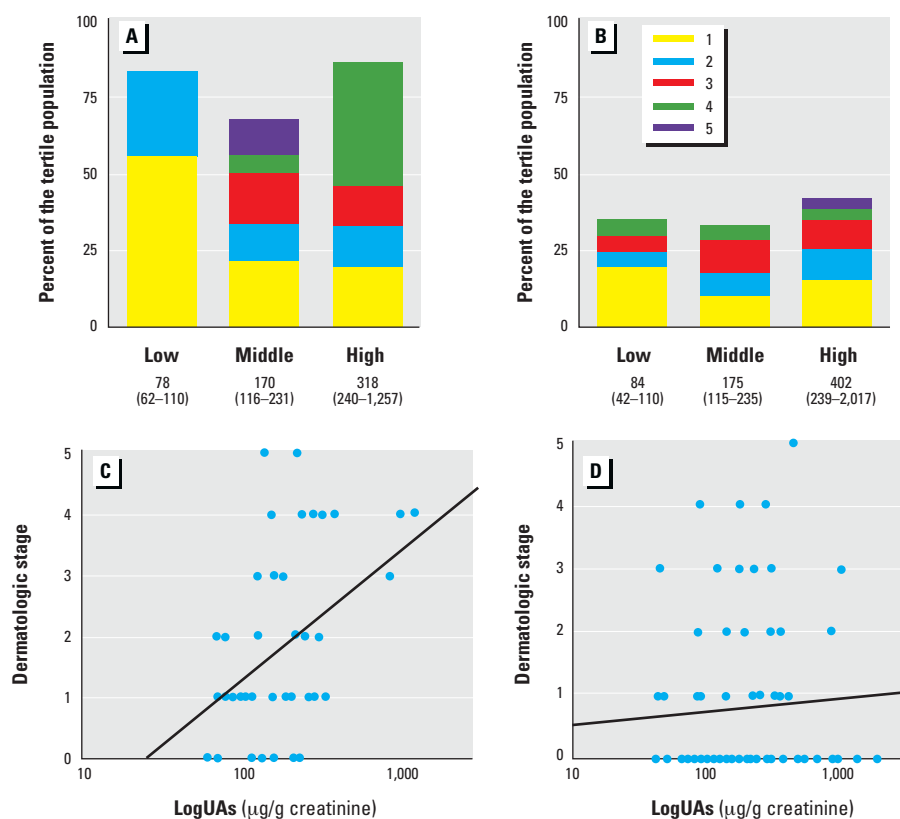

Figure 3. Dose–response relationship at different levels of $[As]_u$ in the SV male and female populations. Male (*A*) or female (*B*) subjects were divided into three tertile groups by the $[As]_u$ values, showing the relative frequency distribution of the positive DS, ranging from 1 (mild) to 5 (most severe), for each tertile group. For males, the distribution pattern differs among the tertiles (log-likelihood chi-square test; $p < 0.05$). In the abscicca, median and range (in parentheses; μg/g creatinine) of the $[As]_u$ for each tertile group are shown. The DS of each male (*C*) or female (*D*) individual was plotted against his/her $[As]_u$. The regression line is arbitrary because the DS is a discrete variable.

overall results of the analyses except for the weakened correlations described above. It should be noted, however, that two previous studies examined morning first-voided urine samples, and both found that creatinine adjustment did not improve the correlation between urinary and environmental arsenic levels (*17,24*). The time of sampling, and therefore the extent of hydration, would be more homogenous in these previous studies than in our study, which may explain the discrepancy.

Urinary arsenic likely reflects only recent exposure to arsenic. The two individuals who changed their tube well between the first and second monitoring events showed drastic changes in $[As]_u$ over the 3-month period. The high correlation between the current $[As]_u$ and $[As]_{tw}$ per se may also be evidence of this phenomenon. Excretion of ingested inorganic arsenic is rapid: more than half of an ingested dose was excreted within 2 days in human experiments (*25–27*). Therefore, it is conceivable that $[As]_u$ reflects recent (up to 1 week) exposure. It should be noted, however, that $[As]_u$ may also reflect past exposure in field situations where there has been chronic exposure (months or years) to arsenic; 2 months after the cessation (substantial reduction) of arsenic exposure via drinking water in a Chilean population, $[As]_u$ remained somewhat higher than the level expected from $[As]_{tw}$ (*24*). The release of arsenic from internal deposits from past exposure at higher levels was thought to be among the possible mechanisms explaining this observation (*24*).

Another noteworthy feature is the large interindividual variation of $[As]_u$, by as much as 10-fold, among individuals sharing the same $[As]_{tw}$ levels, despite a good correlation between $[As]_u$ and $[As]_{tw}$ for the entire

population (or each subpopulation). Visual inspection of the data did not suggest that sex, age, or percent body fat could adequately explain the variation. Within-individual, day-to-day variation was within a 3-fold range (56–265%; Figure 1) and did not appear to account for the difference. Interindividual differences in arsenic intake from sources other than their regular tube well (including food or water from other tube wells) might be a factor in accounting for such variation in $[As]_u$. A visual inspection of the regression line (Figure 2) shows that in the lower $[As]_{tw}$ range (up to ~100 µg/L), $[As]_u$ was much higher than $[As]_{tw}$, suggesting such extra sources of arsenic. Alternatively, some environmental, nutritional (e.g., a minor nutrient such as selenium), or genetic factors may modify the toxicokinetics of ingested arsenic. Another possible source of the variation between individuals is variation in past exposure, as discussed above. These possible reasons for interindividual variation in $[As]_u$ should be tested in future research. A similar range of interindividual variation was reported in a previous study in Bangladesh, which speculated that the differences were due to variation in arsenic metabolism between individuals (*7*). In this context, it bears mentioning that many preceding studies found arsenicosis among the groups consuming the lowest levels of $[As]_{tw}$, which was speculatively ascribed to the putative existence of susceptible subgroups (*7,9,12*). The present results suggest that such differences may have their origins in the kinetics/intake of arsenic, rather than in susceptibility.

Sex differences in the dose–response relationship. The $[As]_u$ values for females were higher than those for males. After correcting for $[As]_{tw}$, the difference was significant. This sex-related difference is probably at least partly due to the creatinine adjustment of the arsenic concentration, by which excretion in females is overestimated relative to that in males (*28*). However, because this would result in an overestimation of 10% at most (*28*), correcting for this factor would reduce or negate the sex-related difference, but never reverse it. Thus, it is unlikely that the exposure level of males exceeded that of females in this population.

In view of the relatively homogenous relationship between $[As]_{tw}$ and $[As]_u$, the distinct differences in the dose–response relationship among subpopulations are noteworthy. The significantly higher median DS in males supports the existence of a sex difference in the dose–response relationship, and the within-household analysis further supports this idea. Relative hyperresponsiveness in males with regard to $[As]_{tw}$ has been described previously (*5,9,12*), but other

studies did not find any sex differences, perhaps partly because of small sample sizes (*5,7*). No study has reported a female hyperresponse. Because the $[As]_u$ levels were either similar between the sexes or were even higher in females, such a sex difference might suggest that males have a higher susceptibility to a given amount of arsenic. Furthermore, the relationship between $[As]_u$ and the rank of each anatomic location (sole, palm, and trunk) showed similar sex-related differences, as males were more susceptible, although statistical significance was not always found (data not shown). More detailed descriptions of sex-differences in the skin manifestations of arsenic toxicity are presented elsewhere (*21*).

There are several alternative interpretations that might argue against higher susceptibility in males. First, the differential coverage rate between sexes (80% of females vs. 50% of males) could have introduced a bias if the males who participated were the males most affected. The within-household comparisons argue against this possibility, as the coverage rate was intrinsically the same. Second, even though the $[As]_u$ levels were similar between the sexes, cumulative exposure may have been much higher in males, who were mostly born in this area, than in females, who mostly migrated from less contaminated areas nearby. The use of tube wells in SV and SP started approximately 20 years ago, at most. Assuming the mean in-migration of females upon marriage occurred at around the age of 20 years, male–female pairs in which the woman is now more than 40 years old would have similar exposure periods. However, even when only such couples were selected, the between-sex difference remained significant when their DS values were compared. Therefore, a sex difference does appear to exist, although the mechanisms that account for the difference are not clear.

Two other potentially confounding factors, sunlight exposure and smoking, may account for the observed sex-related difference in the dose–response relationship. Exposure to sunlight is associated with dermatologic conditions such as melanosis and keratosis (*29*). If such changes are imposed on arsenic-induced skin lesions, then males, being more likely to be exposed to sunlight than females (due to farming activities), may have more severe skin manifestations. However, such a facilitating effect of sunlight is usually confined to sunlight-exposed areas of skin, while the sole of the foot and, to a lesser extent, the palm of the hand, where the skin lesions were diagnosed, are two of the least sun-exposed sites on the body, and melanosis was also prominent in unexposed areas of the trunk. Therefore, it appears unlikely that sunlight had a substantial effect on the skin manifestations
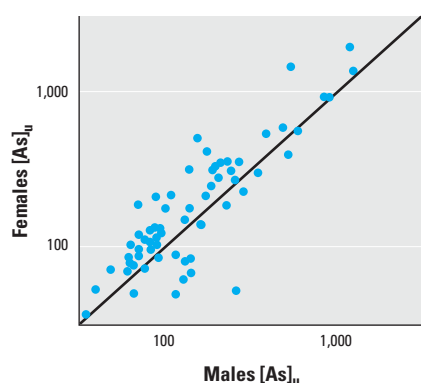

Figure 4. Comparison of $[As]_u$ between males and females. Each circle denotes a couple who shared a tube well. The correlation was highly significant for both areas. The regression line for SP is female $[As]_u$ = 0.188 ± 0.950 (male $[As]_u$; r = 0.88), and for SV is female $[As]_u$ = 0.105 ± 0.987 (male $[As]_u$; r = 0.83). Both lines were statistically significant ($p < 0.001$).

observed, although this factor should not be neglected. The contribution of smoking to the observed sex difference is currently unknown, although smoking was rarely observed, even among males, because most of the subjects in the study area were Muslims, who tend to refrain from smoking.

Finally, it should be added that despite the similar environment and lifestyle shared by the two communities, the dose–response relationship was apparent only in the SV males. This could be a chance finding. Alternately, an unidentified environmental factor (such as minor nutrients) or genetic factors may lead to such a difference. Elucidation of such modifying factors should be of great importance in the future implementation of any mitigation or intervention measures. This requires further investigations of human populations.

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